STN Search History

FILE 'HOME' ENTERED AT 08:17:14 ON 23 SEP 2003

L3 : 65 L1 AND (M2 OR M-2) AND (DELET##### OR REMOV## OR SUBSTITUT####) (S) (TRANSMEMBRANE OR TM)

(FILE 'HOME' ENTERED AT 08:17:14 ON 23 SEP 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 08:17:41 ON 23 SEP 2003

L1 1501 S (INFLUENZ## WITH VIRUS) AND (M2 OR M-2)
L2 1403 S L1 AND M2
L3 65 S L1 AND (M2 OR M-2) AND (DELET##### OR REMOV## OR SUBSTITUT###
L4 22 DUP REM L3 (43 DUPLICATES REMOVED)
L5 2 S L4 AND (GLYCINE OR HYROPHILIC)
L6 20 S L4 NOT L5
L7 11 S L6 NOT PY>1998

L5 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:375676 CAPLUS
DN 131:28629
TI Preparation and use of recombinant **influenza** A **virus** **M2** protein constructs and vaccines
IN Frace, A. Michael; Klimov, Alexander I.; Katz, Jacqueline M.
PA Centers for Disease Control and Prevention, USA
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9928478	A1	19990610	WO 1998-US16379	19980806
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6169175	B1	20010102	US 1997-906930	19970806
	AU 9890150	A1	19990616	AU 1998-90150	19980806
	AU 755475	B2	20021212		
	EP 1002093	A1	20000524	EP 1998-942007	19980806
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002508153	T2	20020319	JP 2000-523354	19980806
	NZ 502398	A	20020426	NZ 1998-502398	19980806
PRAI	US 1997-906930	A	19970806		
	WO 1998-US16379	W	19980806		

AB The present invention provides a method of increasing the expression and soly. of a modified **M2** protein from **influenza** A **virus**, in which **transmembrane** and other hydrophobic domains have been **deleted**. The present invention also provides purified polypeptides encoded by the disclosed nucleic acids, and said polypeptides are immunogenic and are less hydrophobic than full-length **M2**. Also provided are vaccines comprising variants of **M2** expressed in prokaryotic hosts. Further provided are methods of preventing influenza A infection using vaccines comprised of variants of **M2**. Also provided are antibodies raised against the variants of **M2**, and use of such antibodies in diagnosis and treatment of influenza A infections.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:157162 BIOSIS
DN PREV199900157162
TI Cu(II) inhibition of the proton translocation machinery of the **influenza** A **virus** **M2** protein.
AU Gandhi, Chris S.; Shuck, Kevin; Lear, James D.; Dieckmann, Gregg R.; Degrado, William F.; Lamb, Robert A.; Pinto, Lawrence H. (1)
CS (1) Dep. Neurobiol. Physiol., Hogan Hall, Northwestern Univ., 2153 N. Campus Dr., Evanston, IL 60208-3520 USA
SO Journal of Biological Chemistry, (Feb. 26, 1999) Vol. 274, No. 9, pp. 5474-5482.
ISSN: 0021-9258.

DT Article
LA English
AB The homotetrameric **M2** integral membrane protein of **influenza virus** forms a proton-selective ion channel. An essential histidine residue (His-37) in the **M2** **transmembrane** domain is believed to play an important role in the conduction mechanism of this channel. Also, this residue is believed to form hydrogen-bonded interactions with the ammonium group of the anti-viral compound, amantadine. A molecular model of this channel suggests that the imidazole side chains of His-37 from symmetry-related monomers of the homotetrameric pore converge to form a coordination site for transition metals. Thus, membrane currents of oocytes of Xenopus laevis expressing the **M2** protein were recorded when the solution bathing the oocytes contained various transition metals. Membrane currents were strongly and reversibly inhibited by Cu2+ with biphasic reaction kinetics. The biphasic inhibition curves may be explained by a two-site model involving a fast-binding peripheral site with low specificity for divalent metal ions, as well as a high affinity site (Kdiss apprx2 muM) that lies deep within the pore and shows rather slow-binding kinetics (kon = 18.6 +- 0.9 M-1 s-1). The pH dependence of the interaction with the high affinity Cu2+-binding site parallels the pH dependence of inhibition by amantadine, which has previously been ascribed to protonation of His-37. The voltage dependence of the inhibition at the high affinity site indicates that the binding site lies within the **transmembrane** region of the pore. Furthermore, the inhibition by Cu2+could be prevented by prior application of the reversible blocker of **M2** channel activity, BL-1743, providing further support for the location of the site within the pore region of **M2**. Finally, **substitutions** of His-37 by alanine or **glycine** eliminated the high affinity site and resulted in membrane currents that were only partially inhibited at millimolar concentrations of Cu2+. Binding of Cu2+ to the high affinity site resulted in an approximately equal inhibition of both inward and outward currents. The wild-type protein showed very high specificity for Cu2+ and was only partially inhibited by 1 mM Ni2+, Pt2+, and Zn2+. These data are discussed in terms of the functional role of His-37 in the mechanism of proton translocation through the channel.

L7 ANSWER 1 OF 11 MEDLINE on STN
AN 97470961 MEDLINE
DN 97470961 PubMed ID: 9326604
TI A functionally defined model for the **M2** proton channel of **influenza** A **virus** suggests a mechanism for its ion selectivity.
AU Pinto L H; Dieckmann G R; Gandhi C S; Papworth C G; Braman J; Shaughnessy M A; Lear J D; Lamb R A; DeGrado W F
CS Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208-3500, USA.
NC AI-20201 (NIAID)
GM-54616 (NIGMS)
GM-56423 (NIGMS)
+
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Oct 14) 94 (21) 11301-6.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199711
ED Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971124
AB The **M2** protein from **influenza** A **virus** forms proton-selective channels that are essential to viral function and are the target of the drug amantadine. Cys scanning was used to generate a series of mutants with successive **substitutions** in the **transmembrane** segment of the protein, and the mutants were expressed in Xenopus laevis oocytes. The effect of the mutations on reversal potential, ion currents, and amantadine resistance were measured. Fourier analysis revealed a periodicity consistent with a four-stranded coiled coil or helical bundle. A three-dimensional model of this structure suggests a possible mechanism for the proton selectivity of the **M2** channel of **influenza virus**.

L7 ANSWER 2 OF 11 MEDLINE on STN
AN 95065645 MEDLINE
DN 95065645 PubMed ID: 7526533
TI Direct measurement of the **influenza** A **virus M2** protein ion channel activity in mammalian cells.
AU Wang C; Lamb R A; Pinto L H
CS Howard Hughes Medical Institute, Northwestern University, Evanston, Illinois 60208.
NC AI-20201 (NIAID)
AI-31882 (NIAID)
SO VIROLOGY, (1994 Nov 15) 205 (1) 133-40.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199412
ED Entered STN: 19950110
Last Updated on STN: 19960129
Entered Medline: 19941206
AB The **influenza** A **virus M2** integral membrane protein has an ion channel activity which is thought to play an essential role in the uncoating process of **influenza virus** in

infected cells and, for some strains of **influenza virus**
, in maintaining the hemagglutinin in its pH neutral form during transport
through the trans Golgi network. To demonstrate directly that the
M2 protein forms an ion channel in mammalian cells, the **M2**
protein was expressed in CV-1 cells by using an SV40-**M2**
recombinant virus and the whole cell membrane currents were recorded. It
was found that the whole cell current was activated by low pH and
inhibited by the **M2** ion channel-specific blocker, amantadine
hydrochloride. Expression of an altered **M2** protein that
contains a **deletion** of four residues in the
transmembrane domain (**M2**-del28-31) and that when found
in **influenza virus** confers amantadine resistance,
resulted in a current that was activated by hyperpolarization of the
membrane, was pH insensitive, and was resistant to block by amantadine.
The data obtained in mammalian cells for the wild-type **M2** and
M2-del28-31 protein ion channel activities were very similar to
those obtained when using the heterologous oocyte expression system.

L7 ANSWER 4 OF 11 MEDLINE on STN
AN 94149845 MEDLINE
DN 94149845 PubMed ID: 7508997
TI **Influenza** A **virus M2** ion channel protein: a
structure-function analysis.
AU Holsinger L J; Nichani D; Pinto L H; Lamb R A
CS Department of Biochemistry, Molecular Biology and Cell Biology,
Northwestern University, Evanston, Illinois 60208-3500.
NC AI-20201 (NIAID)
AI-31882 (NIAID)
SO JOURNAL OF VIROLOGY, (1994 Mar) 68 (3) 1551-63.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199403
ED Entered STN: 19940330
Last Updated on STN: 19960129
Entered Medline: 19940323
AB A structure-function analysis of the **influenza** A **virus**
M2 ion channel protein was performed. The **M2** protein of
human **influenza virus** A/Udorn/72 and mutants
containing changes on one face of the putative alpha helix of the
M2 transmembrane (TM) domain, several of which lead to amantadine
resistance when found in virus, were expressed in oocytes of Xenopus
laevis. The membrane currents of oocytes expressing mutant **M2**
ion channels were measured at both normal and low pH, and the
amantadine-resistant mutant containing the change of alanine at residue 30
to threonine was found to have a significantly attenuated low pH
activation response. The specific activity of the channel current of the
amantadine-resistant mutants was investigated by measuring the membrane
current of individual oocytes followed by quantification of the amount of
M2 protein expressed in these single oocytes by immunoblotting
analysis. The data indicate that changing residues on this face of the
putative alpha helix of the **M2** TM domain alters properties of
the **M2** ion channel. Some of the **M2** proteins
containing changes in the TM domain were found to be modified by addition
of an N-linked carbohydrate chain at an asparagine residue that is
membrane proximal and which is not modified in the wild-type **M2**
protein. These N-linked carbohydrate chains were further modified by
addition of polylactosaminoglycan. A glycosylated **M2** mutant

protein (**M2** + V, A30T) exhibited an ion channel activity with a voltage-activated, time-dependent kinetic component. Prevention of carbohydrate addition did not affect the altered channel activity. The ability of the **M2** protein to tolerate **deletions** in the **TM** domain was examined by expressing three mutants (del29-31, del28-31, and del27-31) containing **deletions** of three, four, and five residues in the **TM** domain. No ion channel activity was detected from expression of **M2** del29-31 and del27-31, whereas expression of **M2** del28-31 resulted in an ion channel activity that was activated by hyperpolarization (and not low pH) and was resistant to amantadine block. Examination of the oligomeric form of **M2** del28-31 indicated that the oligomer is different from wild-type **M2**, and the data were consistent with **M2** del28-31 forming a pentamer.

L7 ANSWER 5 OF 11 MEDLINE on STN
AN 93338365 MEDLINE
DN 93338365 PubMed ID: 7687902
TI [The molecular mechanism of the action of antiviral preparations in the adamantane series].
Molekuliarnyi mekhanizm deistviia antivirusnykh preparatov adamantanovogo riada.
AU Kiselev O I; Blinov V M; Kozeletskaia K N; Il'enko V I; Platonov V G; Chupakhin O N; Stukova M A; Karginov V A
SO VESTNIK ROSSIISKOI AKADEMII MEDITSINSKIKH NAUK, (1993) (3) 10-5.
Journal code: 9215641. ISSN: 0869-6047.
CY RUSSIA: Russian Federation
DT Journal; Article; (JOURNAL ARTICLE)
LA Russian
FS Priority Journals
EM 199308
ED Entered STN: 19930917
Last Updated on STN: 19970203
Entered Medline: 19930831
AB Regions of possible interaction between remantadine and **transmembrane M2** protein are revealed by analysis of amino acid **substitutions** in remantadine- and deutiforin-resistant **influenza viruses**. The major region includes 5-6 amino acid residues at position 25-31, partially involving the premembrane region and the first position of a hydrophobic membrane-associated domain. The proposed model action of remantadine and its derivatives suggests that remantadine is included into the cell membrane lipid bimolecular layer by its adamantane share and its positively charged NH2-group is exposed to the cell surface. This allows remantadine and its analog to be regarded as molecular "hindrances" for viral particle decapsidation and budding.

L7 ANSWER 6 OF 11 MEDLINE on STN
AN 88188229 MEDLINE
DN 88188229 PubMed ID: 3282079
TI Genetic basis of resistance to rimantadine emerging during treatment of **influenza virus** infection.
AU Belshe R B; Smith M H; Hall C B; Betts R; Hay A J
CS National Institute for Medical Research, The Ridgeway, Mill Hill, London, United Kingdom.
NC N01-A1-52575
SO JOURNAL OF VIROLOGY, (1988 May) 62 (5) 1508-12.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals
OS GENBANK-M20326
EM 198805
ED Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880526
AB The emergence of **influenza** A **viruses** which had acquired resistance to rimantadine during a clinical trial (C. B. Hall, R. Dolin, C. L. Gala, D. M. Markovitz, Y. Q. Zhang, P. H. Madore, F. A. Disney, W. B. Talpey, J. L. Green, A. B. Francis, and M. E. Pichichero, Pediatrics 80:275-282, 1987) provided the opportunity to determine the genetic basis of this phenomenon. Analysis of reassortant viruses generated with a resistant clinical isolate (H3N2) and the susceptible influenza A/Singapore/57 (H2N2) virus indicated that RNA segment 7 coding for matrix and **M2** proteins conferred the resistant phenotype. Resistant viruses isolated from seven patients each contained a single change in the nucleotide sequence coding for the **M2** protein which resulted in **substitutions** in amino acid 30 (two viruses) or 31 (five viruses) in the **transmembrane** domain of the molecule. These changes occurred in locations identified in **influenza viruses** selected for resistance to amantadine in tissue culture and indicate a common mechanism of action of the two compounds in cell culture and during chemotherapeutic use.

L7 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1997:567307 CAPLUS
DN 127:229080
TI Amantadine and rimantadine-mechanisms
AU Hay, Alan J.
CS Division of Virology, National Institute for Medical Research, London, NW7 1AA, UK
SO Antiviral Drug Resistance (1996), 43-58. Editor(s): Richman, Douglas D. Publisher: Wiley, Chichester, UK.
CODEN: 64XZAM
DT Conference; General Review
LA English
AB A review with 51 refs. on the mol. bases of susceptibility and resistance of **influenza** A **viruses** to the actions of amantadine and rimantadine. Amantadine and rimantadine suppress the replication of **influenza** A **viruses** by blocking the proton channel activity of the **M2** protein. Single amino acid **substitutions** within the N-terminal half of the **transmembrane** pore appear to interfere with the interaction of drug with this region of the channel to abrogate an irreversible allosteric block. The effects of these amino acid changes on the ion-conductance and pH-modulating activity of **M2**, as well as its susceptibility to drug, depend on the primary structure of the protein. More extensive structure-activity investigations should provide a clearer understanding of the mechanism of proton transfer and regulation of ion permeation, and the mechanisms of inhibition of **M2** by and resistance to amantadine and rimantadine. This may also prove helpful in developing alternative inhibitors of **M2** and potential drugs against and other virus ion channels.

L7 ANSWER 8 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1993:102337 BIOSIS
DN PREV199395057533
TI Influence of amantadine resistance mutations on the pH regulatory function of the **M2** protein of **influenza** A **viruses**.
AU Grambas, Setareh; Bennett, Michael S.; Hay, Alan J. (1)

CS (1) National Institute Medical Research, Ridgeway, Mill Hill, London NW7 1AA UK
SO Virology, (1992) Vol. 191, No. 2, pp. 541-549.
ISSN: 0042-6822.
DT Article
LA English
AB Mutations in the influenza **M2** membrane protein which confer resistance to the antiviral drug amantadine are exclusively located within the **transmembrane** region of the molecule. The influence of specific amino acid **substitutions** on the activity of the **M2** protein in **influenza** A **virus**-infected cells is assessed in this report by their effects upon haemagglutinin (HA) stability and virus growth. A number of amino acid **substitutions**, e.g., L26H, A30T, S31N and G34E reduced the activity of the **M2** protein of A/chicken/Germany/34 (Rostock) and caused a substantial increase in expression of the low-pH form of HA. The adverse effects of the mutations on virus replication were evident from changes selected during subsequent passage of the mutant viruses in the presence or absence of amantadine: reversion to wt, the acquisition of a second suppressor mutation in **M2**, or the appearance of a complementary mutation in HA which increased its pH stability. In contrast, 127T and 127S, mutations which were most readily selected following 127T mutation suppressed the attenuating effects of the A30T and S31N mutations on **M2** activity. The influence of primary structure on the consequences of particular amino acid changes was further emphasized by the contrasting effects of the G34E mutation on the consequences of particular amino acid changes was further emphasized by the contrasting effects of the G34E mutation on the activities of two closely related proteins, causing an increase in the activity of the **M2** of A/chicken/Germany/27 (Weybridge) as opposed to the decrease in activity of the Rostock protein. Estimates of differences in trans Golgi pH based on the degree of conversion of HA to the low-pH form, or complementation of differences in pH stability of mutant HAs, indicate that changes in **M2** may influence pH within the transport pathway by as much as 0.6. The results thus provide further evidence that **M2** regulates **transmembrane** pH gradients in the trans Golgi. Incompatibility between particular HA and **M2** components and the selection of **M2** mutants with suboptimal activity stresses the essential relationship between the structures and functions of these two virus proteins.

L7 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN.
AN 1992:93373 BIOSIS
DN BA93:49923
TI AMANTADINE SELECTION OF A MUTANT **INFLUENZA VIRUS** CONTAINING AN ACID-STABLE HEMAGGLUTININ GLYCOPROTEIN EVIDENCE FOR VIRUS-SPECIFIC REGULATION OF THE PH OF GLYCOPROTEIN TRANSPORT VESICLES.
AU STEINHAUER D A; WHARTON S A; SKEHEL J J; WILEY D C; HAY A J
CS NATIONAL INST. MED. RES., RIDGEWAY, MILL HILL, LONDON NW7 1AA, UK.
SO PROC NATL ACAD SCI U S A, (1991) 88 (24), 11525-11529.
CODEN: PNASA6. ISSN: 0027-8424.
FS BA; OLD
LA English
AB Mutants of **influenza** Rostock **virus** (H7N1 subtype) were selected for resistance to amantadine hydrochloride at concentrations of the antiviral drug known to affect the function of the virus **M2** **transmembrane** protein. Sequence analysis revealed that three mutants had no changes in **M2** but contained a lysine to isoleucine **substitution** in the hemagglutinin (HA) membrane glycoprotein at position 58 of HA2. The mutant viruses were found to fuse membranes at a pH value 0.7 lower than wild type and to exhibit changes in

the conformation of their HAs specifically at the lower pH. The homologous lysine to isoleucine **substitution** was introduced by site-specific mutagenesis into the HA of X-31 **influenza virus** (H3 subtype), which was expressed by using vaccina virus recombinants. The expressed HA also mediated membrane fusion and changed in conformation at a pH value 0.7 lower than wild type. These results indicate that increased acid stability of the HA obviates the consequences of the inhibition of **M2** function by amantadine and provide further evidence for the role of **M2** in regulating the pH of vesicles involved in glycoprotein transport to the cell surface.

L7 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1988:398395 BIOSIS
DN BA86:71034
TI INTEGRATION OF A SMALL INTEGRAL MEMBRANE PROTEIN **M-2** OF **INFLUENZA VIRUS** INTO THE ENDOPLASMIC RETICULUM ANALYSIS OF THE INTERNAL SIGNAL-ANCHOR DOMAIN OF A PROTEIN WITH AN ECTOPLASMIC AMINO TERMINUS.
AU HULL J D; GILMORE R; LAMB R A
CS DEP. BIOCHEM., MOLECULAR BIOL. AND CELL BIOL., NORTHWESTERN UNIV., EVANSTON, ILLINOIS 60208.
SO J CELL BIOL, (1988) 106 (5), 1489-1498.
CODEN: JCLBA3. ISSN: 0021-9525.
FS BA; OLD
LA English
AB The **M2** protein of **influenza** A **virus** is a small integral membrane protein of 97 residues that is expressed on the surface of virus-infected cells. **M2** has an unusual structure as it lacks a cleavable signal sequence yet contains an ectoplasmic amino-terminal domain of 23 residues, a 19 residue hydrophobic **transmembrane** spanning segment, and a cytoplasmic carboxyl-terminal domain of 55 residues. Oligonucleotide-mediated **deletion** mutagenesis was used to construct a series of **M2** mutants lacking portions of the hydrophobic segment. Membrane integration of the **M2** protein was examined by in vitro translation of synthetic mRNA transcripts prepared using bacteriophage T7 RNA polymerase. After membrane integration, **M2** was resistant to alkaline extraction and was converted to an Mr .apprxeq. 7,000 membrane-protected fragment after digestion with trypsin. In vitro integration of **M2** requires the cotranslational presence of the signal recognition particle. **Deletion** of as few as two residues from the hydrophobic segment of **M2** markedly decreases the efficiency of membrane integration, whereas **deletion** of six residues completely eliminates integration. **M2** proteins containing **deletions** that eliminate stable membrane anchoring are apparently not recognized by signal recognition particles, as these polypeptides remain sensitive to protease digestion, indicating that in addition they do not have a functional signal sequence. These data thus indicate that the signal sequence that initiates membrane integration of **M2** resides within the **transmembrane** spanning segment of the polypeptide